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Short communication

Determination of nadoxolol in human plasma using reversed-phase high-performance liquid chromatography

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Abstract

A rapid, simple and accurate HPLC method is presented for the determination of nadoxolol in human plasma. Nadoxolol from plasma was successfully purified using an Adsorbex column. The samples were chromatographed on a LiChrosorb RP-18 (10 μm) column with methanol–acetonitrile–phosphate buffer (pH 3.3) (70:20:10) as the mobile phase. Detection was carried out at 254 nm. The method was tested for linearity (from 5 to 25 μg/ml), recovery (85%) and precision (C.V. = 4.5%).

1. Introduction

Nadoxolol, 3-hydroxy-4-(1-naphthyloxy)butyramide oxime, is used in several European countries for the suppression of cardiac arrhythmias. It exerts a stabilizing activity on membranes of heart cells and has a better therapeutic index and prolonged action compared with quinidine [1]. There have been no reports on the determination of nadoxolol in biological fluids.

This paper describes a rapid and accurate HPLC method for the determination of nadoxolol plasma concentrations.

2. Experimental

2.1. Apparatus

A Type 302 liquid chromatograph with a fixed-wavelength UV 254 nm detector (Techma-Robot, Warsaw, Poland) was used. The analytical column was 20 cm × 4 mm I.D., packed with LiChrosorb RP-18, particle size 10 μm (Merck, Darmstadt, Germany). A Type 327 reciprocating shaker (Premed, Marki, Poland) and a K 24 D high-speed refrigerated centrifuge (MLW, Engelsdorf, Germany) with 11–30-ml propylene centrifuge tubes were applied. The columns for solid-phase extraction were Adsorbex (Merck), packed with 400 mg of LiChrosorb RP-18.

2.2. Reagents and chemicals

Nadoxolol hydrochloride was obtained from Laboratoire L. Lafon (Maison-Alfort, France) and analytical-reagent or reagent-grade diethyl ether, acetonitrile, anhydrous ethanol, methanol, phosphoric acid, sodium hydroxide and monobasic potassium phosphate from Factory of Chemical Reagents (Gliwice, Poland) and Merck.

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Heparinized human whole blood was obtained from the District Blood Centre in Lublin (Poland). A stock standard methanolic solution of nadoxolol hydrochloride (100 μg/ml of free base) was prepared.

2.3. Chromatographic conditions and sample preparation

The mobile phase was methanol–acetonitrile–phosphate buffer (pH 3.3) (0.067 *M* potassium dihydrogenphosphate adjusted to pH 3.3 with phosphoric acid; pH tolerance ±0.1) (7:2:1, v/v). The flow-rate of the mobile phase was 1 ml/min and the detector output range was 0.05 AUFS.

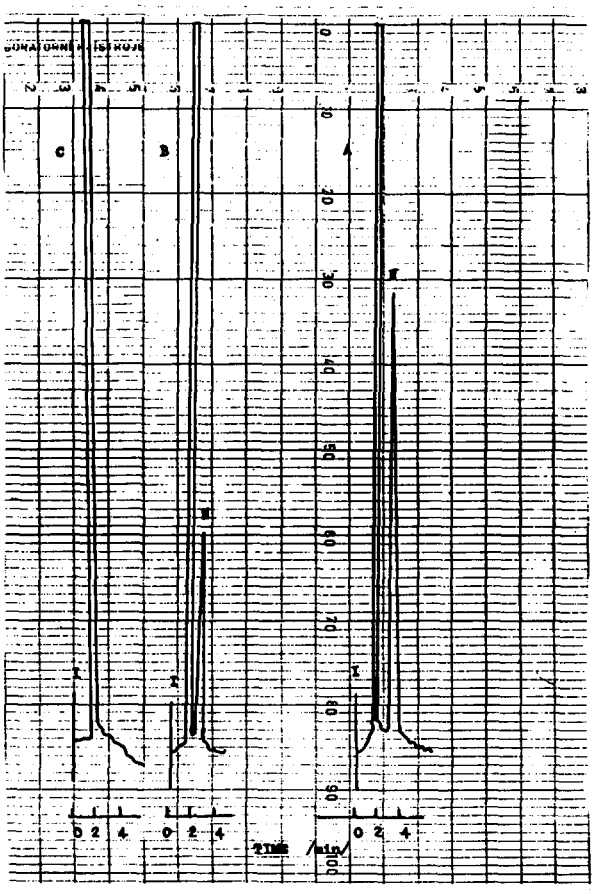

Fig. 1. Chromatograms of nadoxolol on LiChrosorb RP-18 (200 × 4 mm I.D.) with detection at 254 nm. Mobile phase, methanol–acetonitrile–phosphate buffer (pH 3.3) (7:2:1); flow-rate, 1 ml/min; AUFS, 0.05. (A and B) spiked plasma samples with different nadoxolol (N) amounts (0.54 and 0.25 μg, respectively); (C) extracted sample blank plasma. I = injection.

For the isolation of nadoxolol from plasma 0.10–0.50 ml of the stock standard solution of nadoxolol were added to test-tubes each containing 0.5 ml of blood plasma and diluted with methanol to a final volume of 1.0 ml. The mixtures were centrifuged for 15 min at 1100 *g* and then 0.5 ml of the supernatants were injected into Adsorbex extraction columns that had previously been rinsed with two 0.5-ml volumes of hexane and then two 1-ml volumes of methanol. Sample solutions were passed through the column at a flow-rate 50 μl/min and 50 μl of each solution were injected into the analytical column. Analyses were performed in duplicate and the mean result was taken.

3. Results and discussion

Nadoxolol, which is highly polar, is not extractable by commonly used organic solvents. Adsorbex columns were applied successfully to the isolation of nadoxolol from plasma. The liquid–solid extraction system provides a separation mechanism that is more selective, efficient and faster than that associated with liquid–liquid partitioning [2].

Nadoxolol added to plasma in methanolic solution is virtually unretained by the sorbent of the column, but interfering components of plasma are retained.

In the range 5–25 μg/ml of nadoxolol a linear relationship between peak height and drug concentration was obtained. The parameters of the calibration graph were $y = 0.01364x + 0.21$ (x = concentration of nadoxolol in μg per 50 μl and y = peak height of nadoxolol in mm) with $r = 0.9996$. The recovery of nadoxolol through the extraction procedure assessed by measuring the peak height of nadoxolol prepared from a blood extract, at concentrations of 5, 10 and 25 μg/ml, with respect to that from a non-extracted standard were found to be 84.21% ($n = 5$), 84.73% ($n = 5$) and 86.30% ($n = 5$) (respectively).

The precision, as the within-day coefficient of variation (C.V.) was 4.9% at 5 μg/ml, 4.5% at 10 μg/ml and 4.3% at 25 μg/ml; the day-to-day C.V. was 9.3%, 8.3% and 7.6% (respectively) ($n = 6$ in each instance).

The detection limit was 1 μg/ml. The retention time for nadoxolol was 2.4 min. An example of the HPLC of nadoxolol is shown in Fig. 1.

The described method makes it possible to determine the concentration of nadoxolol in the expected therapeutic ranges as the drug (Bradyl tablets) is commonly administered as a single dose of 0.25 g, with a daily dose from 0.75 to 1.5 g.

Acknowledgement

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References

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